Supplementary Materials for

Gut-Liver physiomimetics reveal paradoxical modulation of IBD-related inflammation by short-chain fatty acids

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This file includes:

Supplementary figures

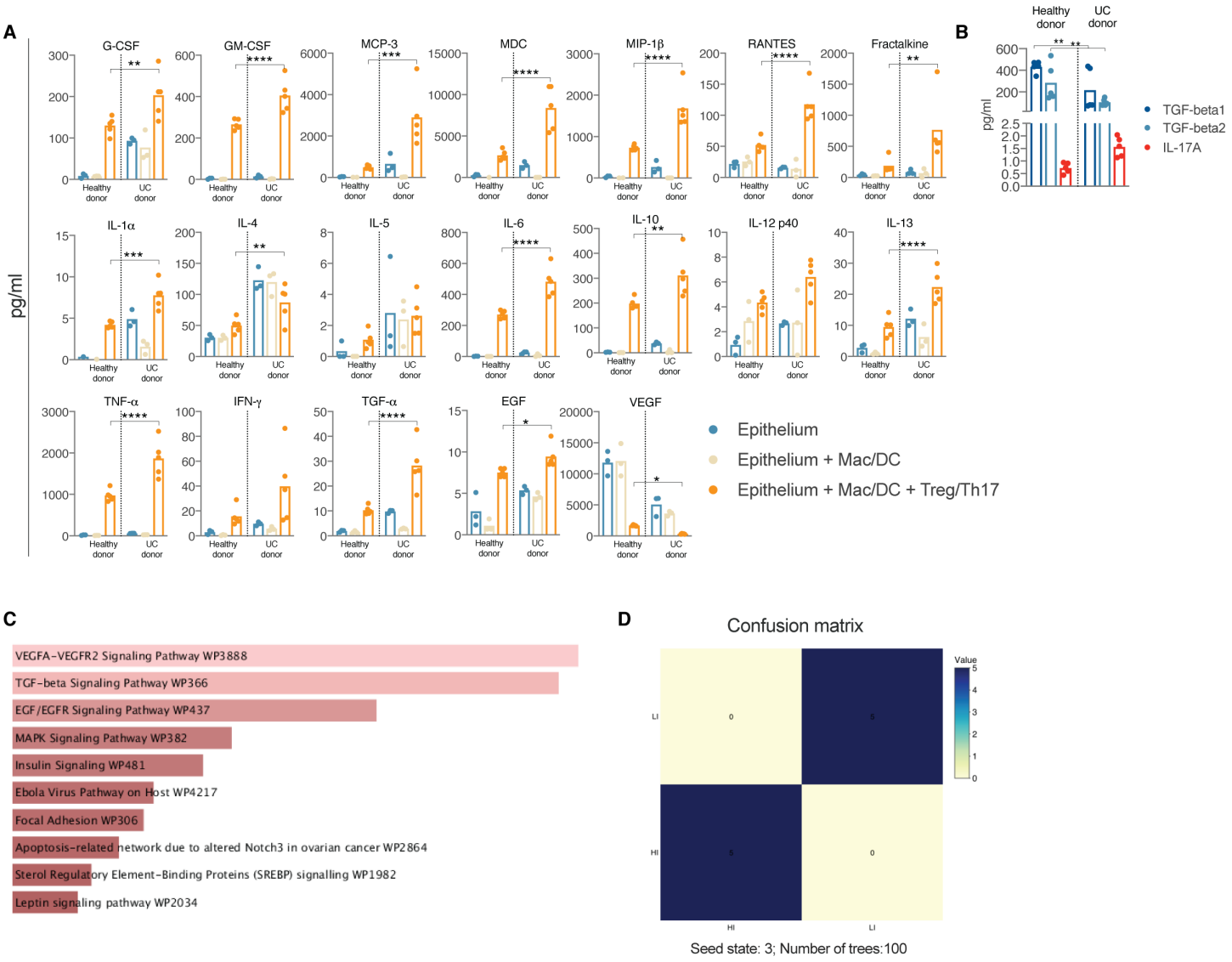


Fig.S1

Gut donor of each MPS can be predicted with 100% accuracy by random forest analysis based on cytokines released during gut MPS-Treg/Th17 interaction. (A) Representative values of individual cytokines measured in media of UC and healthy epithelial monolayers, UC and healthy gut MPS and MPS co-cultures with Treg/Th17 cells (B) Concentration of TGF-β1, TGF-β2 and IL-17 in basal media of UC and helthy gut MPS during interaction with Treg/Th17 cells (A-B) *FDR<0.05; **FDR<0.01; ***FDR<0.001; ****FDR<0.0001. (C) List of top 10 differential gene expression pathway enrichments, ranked based on combined z and p score, by Wikipathway analysis of UC gut MPS over healthy control (D) Random forest analysis of cytokines shown under (A) during UC and helthy MPS interaction with Treg/Th17 cells. Confusion matrix indicates prediction accuracy. Each condition was tested in separate experiments with 3-5 biological replicates.

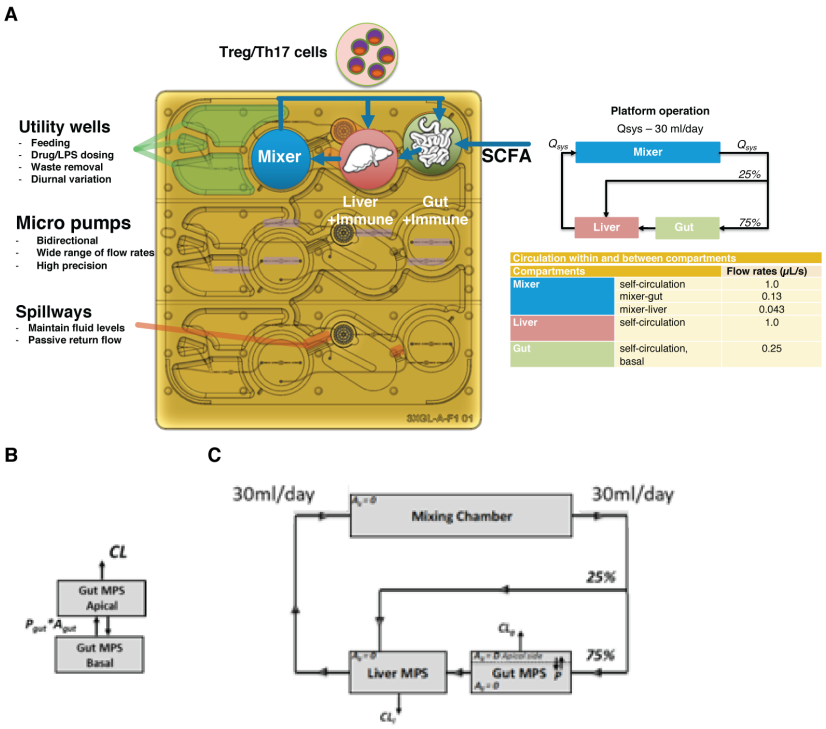
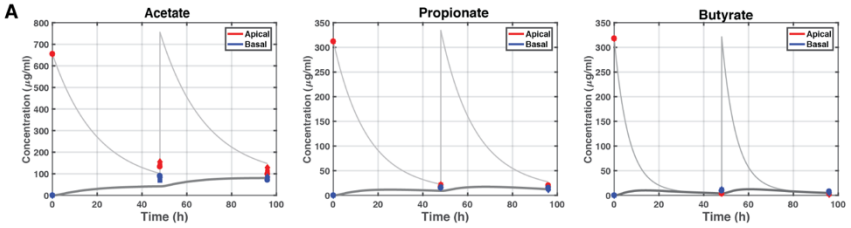
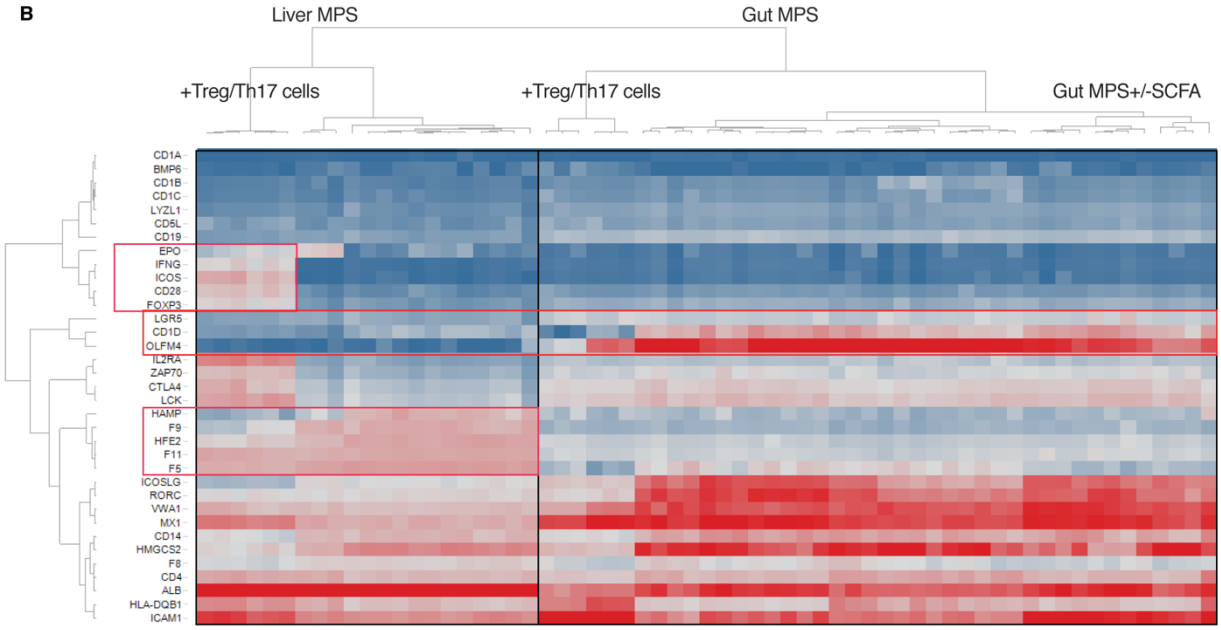


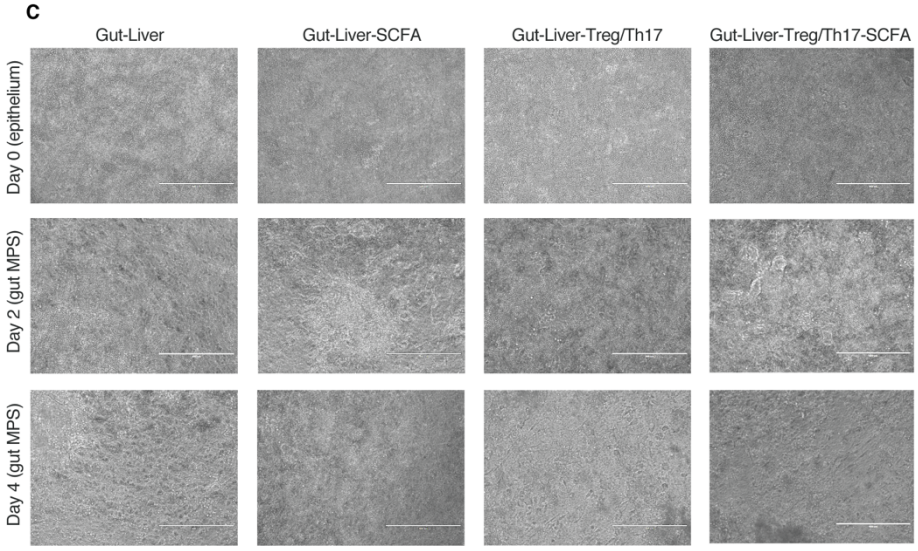
Fig.S2

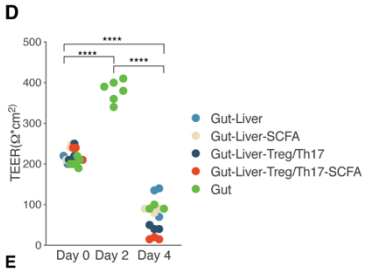
Platform operation, circulating CD4 T cell viability and schematic models to calculate SCFA metabolism and distribution. (A) Operational parameters of the 3XGL platform with the gut and liver MPSs. (B) Schematic overview of the utilized compartmental model to describe SCFA distribution, metabolism in the gut MPS; P_{gut} = permeability, A_{gut} = surface area of transwell, CL = metabolism. (C) Schematic overview of the four-comparmental model consisting of a gut, liver and mixing chamber MPS. In addition to gut-specific SCFA permeability and consumption, liver metabolism was implemented to describe the distribution.



Parameter	Clearance liver (E-5*ml/min)
Acetate	36
Propionate	235
Butyrate	321







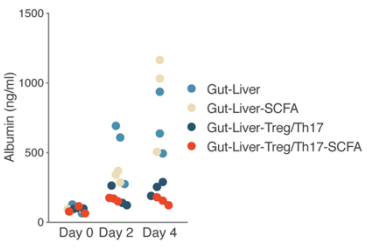


Fig.S3

SCFA distribution and its effect on global gene expression and functional parameters of gut and liver function during gut-liver interaction with or without SCFA and Treg/Th17 cells. (A) Apical concentrations of acetate, propionate and butyrate in UC gut MPS and their bioavailable concentrations in basal common medium during 96h of interaction. Far right: hepatic clearance of bioavailable SCFA. (B) Complete linkage and cluster heatmap of genes expressed by all tissues collected during the study. (C) Representative brightfield images of epithelial monolayers at day 0 of interaction, prior to basolateral seeding of MACs/DCs, and that of gut MPS monolayers with underlying MACs/DCs at day 2 and day 4 of interaction (D) TEER of UC gut MPS used in the interaction studies as well as the control. D0: at the beginning of the interaction, D2: TEER of the control gut MPS in isolation, D4: end of the interaction. ****FDR<0.0001. (E) Albumin concentrations produced by the liver MPS before, during and at the end of interaction measured in common medium.

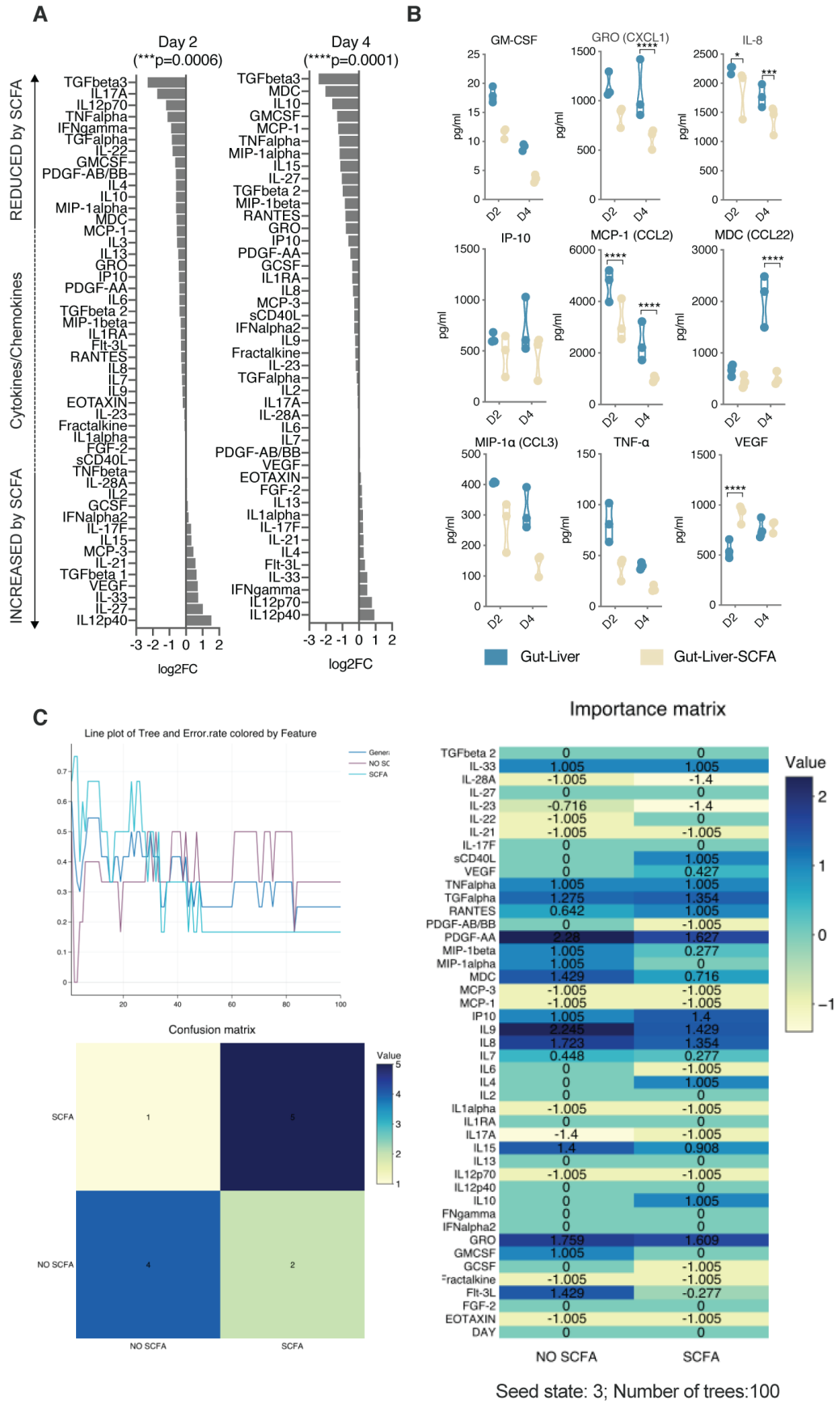

A
Day 2
(***p=0.0006)
Day 4
(****p=0.0001)
REDUCED by SCFA
Cytokines/Chemokines
INCREASED by SCFA
log2FC
B
GM-CSF
GRO (CXCL1)
IL-8
IP-10
MCP-1 (CCL2)
MDC (CCL22)
MIP-1α (CCL3)
TNF-α
VEGF
pg/ml
D2
D4
Gut-Liver
Gut-Liver-SCFA
C
Line plot of Tree and Error.rate colored by Feature
Confusion matrix
SCFA
NO SCFA
Value
Importance matrix
Seed state: 3; Number of trees:100

Fig.S4

Effect of SCFA on cytokine/chemokine expression during gut-liver interaction in the absence of Treg//Th17 cells. (A) log2 Fold changes of individual multiplexed cytokines/chemokines measured in the common medium of the gut-liver and gut-liver-SCFA interactions. p values indicate 2-Way ANOVA difference between SCFA treated and non-treated groups. (B) Concentrations of cytokines/chemokines identified by random forest analysis to be most predictive of condition. *FDR<0.05; **FDR<0.01; ***FDR<0.001; ****FDR<0.0001. (C) Random forest analysis of all cytokines/chemokines measured. Top left: Number of trees required to complete confusion matrix. Bottom left: Confusion matrix indicating prediction accuracy. Right: Importance matrix of most predictive parameters.

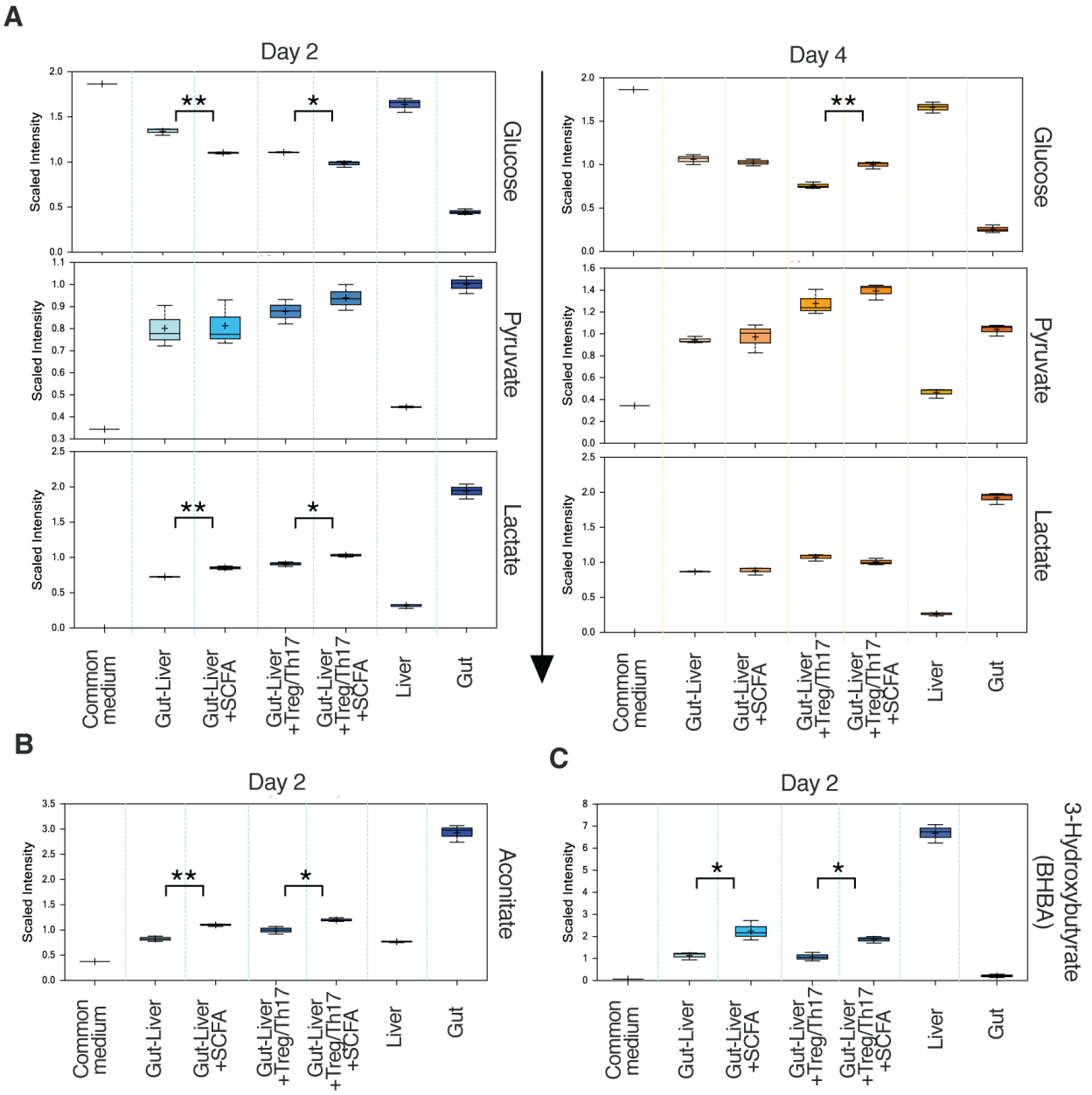


Fig.S5
SCFA and systemic inflammation affect glucose metabolism and production of ketone bodies. (A-C) Scaled intensities of glucose, pyruvate and lactate at days 2 and 4 (A), aconitate (B), and BHBA (C) at day 2 for interactions among all 4 experimental conditions, MPS controls in isolation and common media. *p<0.05; **p<0.01.

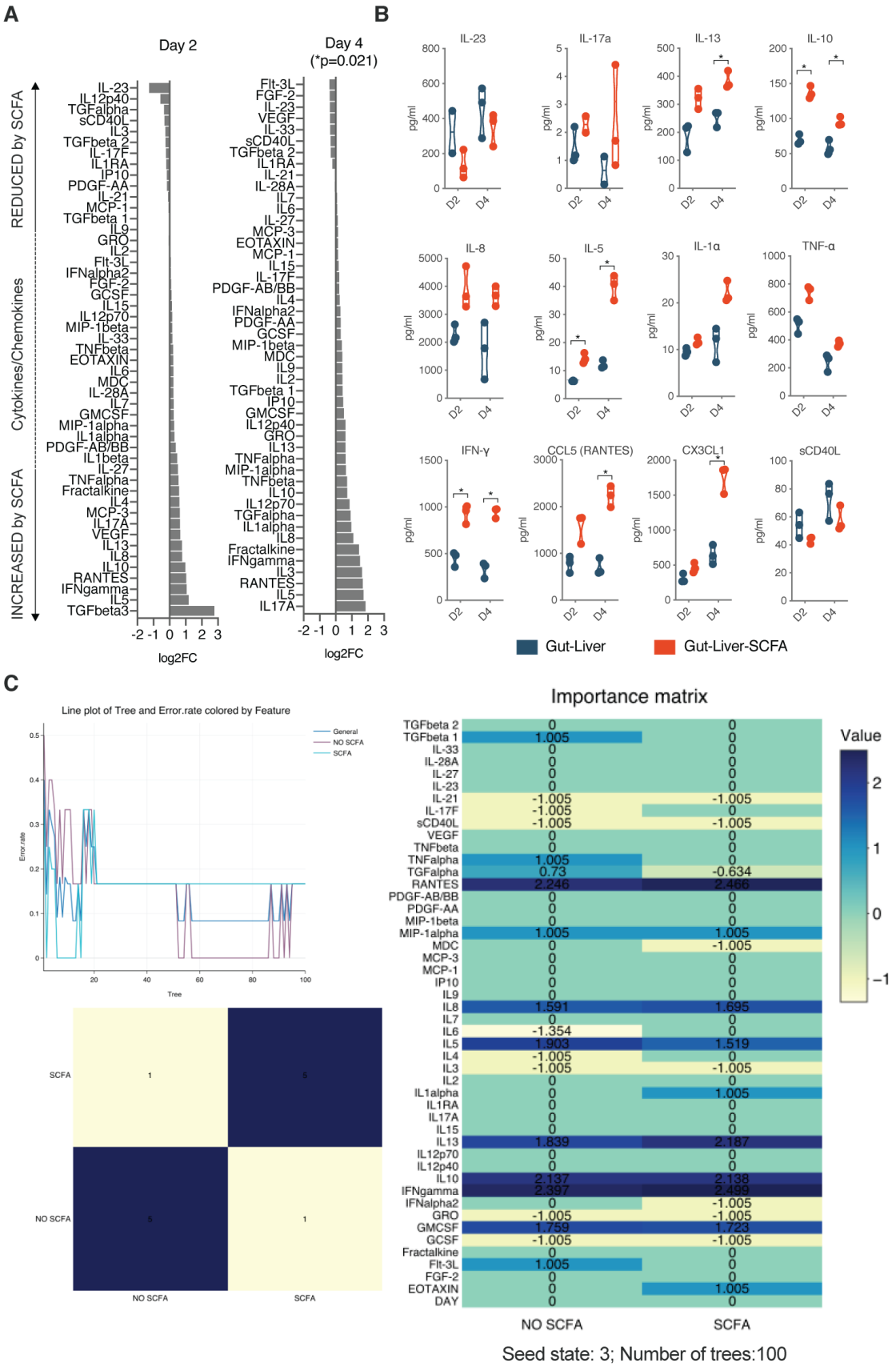

A
Day 2
Day 4
(*p=0.021)
REDUCED by SCFA
Cytokines/Chemokines
INCREASED by SCFA
IL-23
IL12p40
TGFalpha
sCD40L
IL3
TGFbeta 2
IL-17F
IL1RA
IP10
PDGF-AA
IL-21
MCP-1
TGFbeta 1
IL9
GRO
IL2
Flt-3L
IFNalpha2
FGF-2
GCSF
IL15
IL12p70
MIP-1beta
IL-33
TNFbeta
EOTAXIN
IL6
MDC
IL-28A
IL7
GMCSF
MIP-1alpha
IL1alpha
PDGF-AB/BB
IL1beta
IL-27
TNFalpha
Fractalkine
IL4
MCP-3
IL17A
VEGF
IL13
IL8
IL10
RANTES
IFNgamma
IL5
TGFbeta3
-2 -1 0 1 2 3
log2FC
Flt-3L
FGF-2
IL-23
VEGF
IL-33
sCD40L
TGFbeta 2
IL1RA
IL-21
IL-28A
IL7
IL6
IL-27
MCP-3
EOTAXIN
MCP-1
IL15
IL-17F
PDGF-AB/BB
IL4
IFNalpha2
PDGF-AA
GCSF
MIP-1beta
MDC
IL9
IL2
TGFbeta 1
IP10
GMCSF
IL12p40
GRO
IL13
TNFalpha
MIP-1alpha
TNFbeta
IL10
IL12p70
TGFalpha
IL1alpha
IL8
Fractalkine
IFNgamma
IL3
RANTES
IL5
IL17A
-2 -1 0 1 2 3
log2FC
B
IL-23
IL-17a
IL-13
IL-10
IL-8
IL-5
IL-1α
TNF-α
IFN-γ
CCL5 (RANTES)
CX3CL1
sCD40L
pg/ml
D2
D4
Gut-Liver
Gut-Liver-SCFA
C
Line plot of Tree and Error.rate colored by Feature
General
NO SCFA
SCFA
Error.rate
Tree
SCFA
NO SCFA
1
5
5
1
NO SCFA
SCFA
Importance matrix
Value
TGFbeta 2 0 0
TGFbeta 1 1.005 0
IL-33 0 0
IL-28A 0 0
IL-27 0 0
IL-23 0 0
IL-21 -1.005 -1.005
IL-17F -1.005 0
sCD40L -1.005 -1.005
VEGF 0 0
TNFbeta 0 0
TNFalpha 1.005 0
TGFalpha 0.73 -0.634
RANTES 2.246 2.466
PDGF-AB/BB 0 0
PDGF-AA 0 0
MIP-1beta 0 0
MIP-1alpha 1.005 1.005
MDC 0 -1.005
MCP-3 0 0
MCP-1 0 0
IP10 0 0
IL9 0 0
IL8 1.591 1.695
IL7 0 0
IL6 -1.354 0
IL5 1.903 1.519
IL4 -1.005 0
IL3 -1.005 -1.005
IL2 0 0
IL1alpha 0 1.005
IL1RA 0 0
IL17A 0 0
IL15 0 0
IL13 1.839 2.187
IL12p70 0 0
IL12p40 0 0
IL10 2.137 2.138
IFNgamma 2.397 2.499
IFNalpha2 0 -1.005
GRO -1.005 -1.005
GMCSF 1.759 1.723
GCSF -1.005 -1.005
Fractalkine 0 0
Flt-3L 1.005 0
FGF-2 0 0
EOTAXIN 0 1.005
DAY 0 0
NO SCFA
SCFA
Seed state: 3; Number of trees:100

Fig.S6

Effect of SCFA on cytokine/chemokine expression during gut-liver interaction in the presence of Treg//Th17 cells (A) log2 Fold changes of individual multiplexed cytokines/chemokines measured in the common medium of the gut-liver-Treg/Th17 and gut-liver-Treg/Th17-SCFA interactions. p values indicate 2-Way ANOVA difference between SCFA treated and non-treated groups. (B) Concentrations of cytokines/chemokines identified by random forest analysis to be most predictive of condition. *FDR<0.05; **FDR<0.01; ***FDR<0.001; ****FDR<0.0001. (C) Random forest analysis of all cytokines/chemokines measured. Top left: Number of trees required to complete confusion matrix. Bottom left: Confusion matrix indicating prediction accuracy. Right: Importance matrix of most predictive parameters.

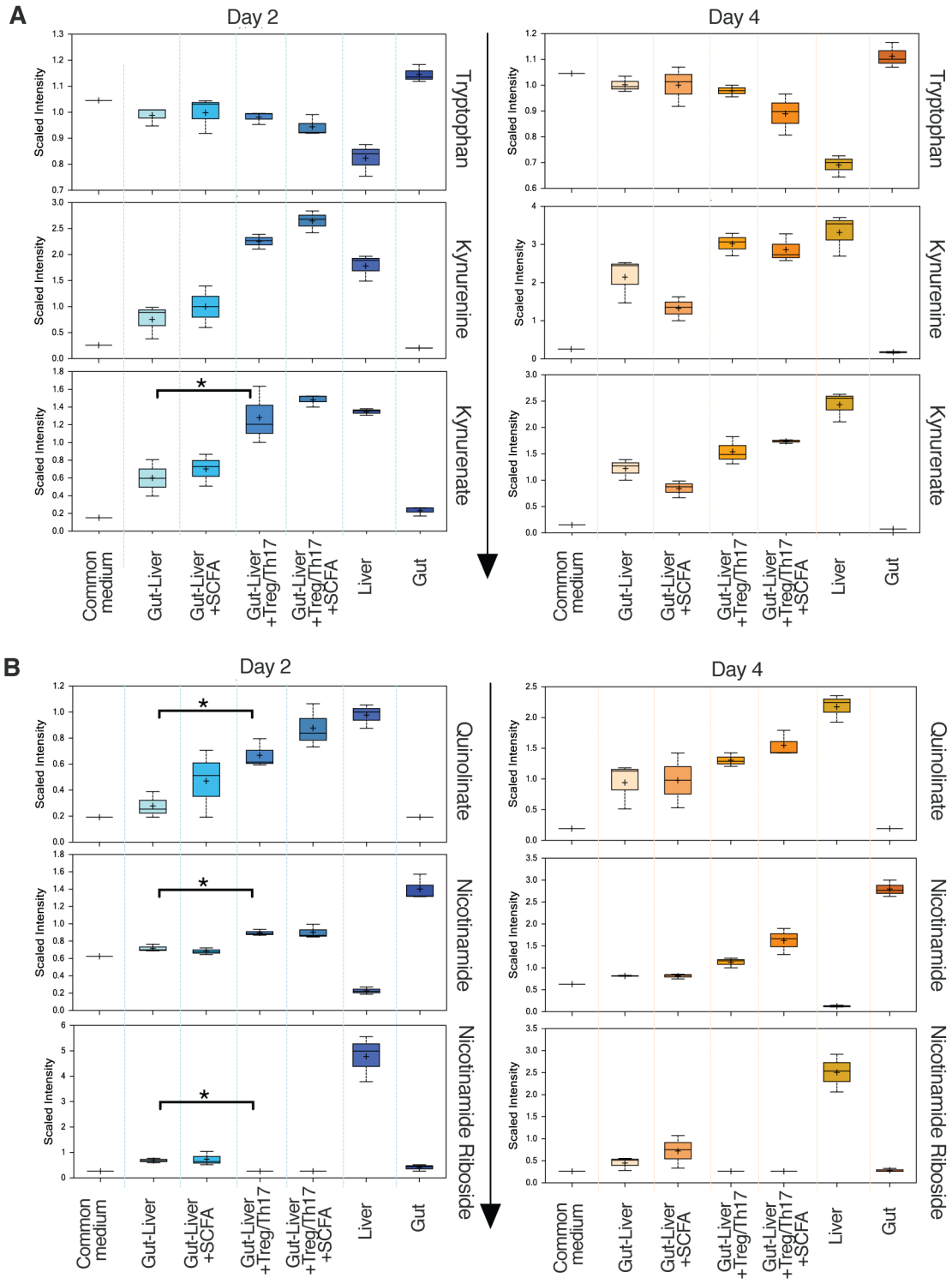


Fig.S7
Effect of SCFA on inflammation related metabolic products during gut-liver interaction (A,B) Scaled intensities of tryptophan, kynurenine and kynurenate (A) as well as quinolinate, nicotinamide and nicotinamide riboside (B) at days 2 and 4 for interactions among all 4 conditions, MPS controls in isolation and common media. *p<0.05; **p<0.01.

Supplementary tables

Table S1. Overview of model parameters

Parameter name	Acronym	Value	Unit
Permeability	P_{gut}	Estimated	cm/min
Surface area	A_{gut}	0.33	cm^2
Metabolism gut	Cl_{gut}	Estimated	ml/min
Metabolism liver	Cl_{liv}	Estimated	ml/min
Media volume gut	V_{apical}	0.5	ml
	V_{basal}	1.5	ml
Media volume liver	V_{liv}	1.6	ml
Media volume mixer	V_{mix}	2.5	ml
Systemic flow rate	Q_{mix}	30	ml/day
Flow partitioning gut	Q_{gut}	$0.75*Q_{mix}$	ml/day
Flow partitioning liver	Q_{hep}	$0.25*Q_{mix}$	ml/day